

THE EFFECTS OF DISTURBANCE AND ENERGY BUDGET,  
INTRINSIC AND ENVIRONMENTAL FACTORS, AND DENSITY ON ANTLION PIT SIZE  
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Term Paper for Behavioral Ecology (EEB 492)  
University of Michigan Biological Station  
15 August 2009

Disclaimer: This was a collaborative effort. My part included the experiments on disturbance and energy budget, including the methods and results sections, as well as the synthesis of the discussion.

## ABSTRACT

Antlions (*Myrmeleon immaculatus*) are sit-and-wait predators who rely on the construction of a conical pit to trap their prey. Several explanations for the determination of pit size have been suggested, ranging from the average temperature of a region to the size of the particles of substrate in which the antlion chooses to build its pit. In this experiment, the effects of disturbance and energy budget, intrinsic versus environmental factors, and density on pit size, measured in average pit diameter, were studied. Antlions were weighed and average pit diameters were measured. The disturbance group was established in deli dishes in the lab and disturbed by filling their pits with sand according to a schedule. Weights and average pit diameter were recorded. In the disturbance experiment, larger antlions built smaller pits than smaller antlions and also lost more weight in the process. In examining energy budget, antlions were set up on different routines of exercise and feeding to establish different energy budgets. Energetic budget does not appear to play an important role in determining pit size. The energy budget experiment revealed no significant difference in weight loss between high energy budget antlions and low energy budget antlions. The undisturbed antlions maintained a larger pit size than those whose pits were disturbed every other day, suggesting that the rate of disturbance affects pit size more so than energy available. A common garden was established in order to test whether environment, genetics, or learning played a role in antlion pit construction. In the intrinsic factors versus environmental experiment, environmental factors were not supported as an explanation for pit size, as Sturgeon Bay antlions built consistently larger pits than Douglas Lake antlions. Genetics were discounted as the sole factor determining pit size as well, with antlions from both Sturgeon Bay and Douglas Lake building the same size pits in a laboratory setting. This suggests an interaction between genetic factors and environmental conditions. Different densities were established in a set area in order to test the density hypothesis. Average pit diameter and nearest neighbor distances were analyzed. Density did have an effect on pit size. Pit sizes in more dense situations increased significantly and nearest neighbor distances became more and more uniform as density increased. Disturbance, intrinsic factors (possibly learning), and density all played considerable roles in determining pit size.

## INTRODUCTION

Different insects display different foraging techniques. Each technique has costs and benefits related to both energy and time; therefore, a tradeoff between maximizing energy and minimizing time must be addressed (See Figure 1). Sit-and-wait predators maximize their energy intake through energy preservation, choosing to wait for prey to encounter the predator instead of actively seeking out prey. Though this technique is effective in preserving energy, an associated cost of time exists because the encounter rate with prey items is not guaranteed. Sit-and-wait predators may withstand long periods between meals, relying solely on their energy stores. Examples of sit-and-wait predators include web-spinning spiders, and antlions.

In studies of web-spinning spiders, the energetic costs of creating a trap were investigated using a Gilson respirometer. It was concluded that, though a large initial investment of energy is associated, the maintenance costs of the web were fairly low and the energetic income fairly high, suggesting that the sit-and-wait predation strategy was energetically effective (Ford, 1977). We expanded these studies of energy effectiveness to a new sit-and-wait insect predator: antlions, as well as investigating what determines the size of an individual's trap.

Antlions (a species of insect in the order Neuroptera), are a part of the family *Myrmeleontidae*, consisting of over 2,000 species. Since these insects are classified as

holometabolists, they complete four life stages (egg, larvae, pupae and adult) and undergo full metamorphosis. In their larval stage, most species of antlions are “sit-and-wait” predators, burrowing themselves into the ground and digging conical pits in which they trap and consume terrestrial insects (typically ants, though any soft-bodied insect able to be handled will suffice). Antlions average in weight from 20-90mg and create pits ranging from 10-90mm in diameter at the bottom of which the antlion will lay with all but its head and pincers submerged under the sand (Deventak, 2005). The antlion builds the pit by flinging sand with its head out from a central point until the sand sits at the angle of repose.

While this strategy of predation has proved to be an effective means of survival, the construction of the pit itself requires high levels of energy. In a previous study on antlion metabolism, it was found that the larva’s metabolic expenditure during the process of pit construction was ten times the metabolic resting rate (Lucas, 1985). Due to this extremely high necessary energy expenditure, it would seem that one could make predictions on pit characteristics by using a relatively simple cost-benefit analysis. Since pit building requires considerable energy from the individual antlion, it can safely be assumed that an unavoidable tradeoff exists regarding the allocation of energy stores, which the animal can apportion to body maintenance or pit construction. After completing a pit, antlion larvae are able to reserve their remaining energy for basal metabolic needs, thus discouraging them from ever making unnecessary pit size adjustments or renovations. In fact, the only time the larvae would ever expend the energy to modify their pits is if an external agent damages the pit, impairing its effectiveness. In applying this logic, it can be hypothesized that a positive correlation exists between number of antlion pit disturbances and energy loss, and that this drop in energy can manifest itself in one of two ways: decreased pit size or weight loss. In using this model, one necessary assumption is that, within the ant-lion community, for a given size of larva, an optimal pit size incurs the highest success rate (Griffiths, 1986). Since the antlion’s livelihood depends entirely on the effectiveness of its pit, we may also assume that the larvae will only deviate from this optimal pit size if in situations of unusually high-energy cost or abnormally low return (ant feedings).

In this experiment, we test the former of these two reasons; predicting that pit size in antlions (*Myrmeleon immaculatus*) will decrease as the number of destructive disturbances increases, due to decreased pit maintenance. Evidence to support our hypothesis can be gleaned from prior experiments conducted on the correlation between pit size and subsequent metabolic rates. In one particular study, antlion larvae metabolic rates were determined for both resting periods and “pit constructing” periods by using a Gilson differential respirometer to assess the rate of oxygen consumption. The study concluded that the total metabolic cost of the pit’s construction is not only a direct result of the construction time (cost per unit time multiplied by the time required to build the pit), but that the cost is also indirectly affected by environmental factors such as temperature, current larval instar stage, and species (Lucas, 1985). Instead of recognizing these outstanding factors as significant players in determining energy costs, we controlled for them in our experiment by standardizing each of the variables in the experimental subjects. We also hypothesized that weight loss would increase with increased disturbance according to three separate models. The first model suggested that the antlions would continue to build their ideal pit regardless of the energy budget required to do so. The second model suggested that the antlions would sacrifice weight to a certain threshold, at which point they would stop making their optimal pit size and would revert to a smaller pit. The third model

assumed that only antlions with a large energy budget would create the optimally sized pit, therefore losing the most energy in doing so (See Figure 2).

In a second experiment, we established a common garden in order to determine whether pit size had an intrinsic component. If antlions from different sites would establish different pit sizes depending on sites, then the environment would have more to do with the determination of pit size than genetics. If they established the same pit size regardless of environment, it could be deduced that pit size is either genetic, a learned behavior, or could be due to physiological conditioning. The results of the first portion of the experiment suggested that intrinsic factors were at work, so a second common garden was created in the laboratory to determine if the antlions would continue building the same size pit in an entirely different environment.

Antlions are strongly thigmotactic, being able to detect the presence of prey in their pit as well as the construction of pits of nearby antlions (McClure, 1976; Simberloff, 1978). This opens the debate as to the observed patterns of both pit construction and size. One hypothesis suggests that antlions survey the surroundings and arrange themselves to optimize prey capture (Wilson, 1974; McClure, 1976). In non-homogeneous environments, clumping patterns may also suggest that antlions construct pits in favorable areas (Simberloff, 1978). A third hypothesis proposes that antlions avoid each other during pit construction and routine maintenance (Matsura, 1989; Griffiths, 1991, 1992).

At low densities, pits are randomly distributed within favorable areas (McClure, 1976), thereby lessening the impacts of peer interference. However, high-density situations result in a higher degree of proximity to neighbors. This proximity results in more sand being within flinging distance of other pits, making for more maintenance required to maintain optimal pit size. In high-density situations, a reduction in the number of pits constructed and a more uniform distribution have been observed (Matsura, 1989; Griffiths, 1991).

The third branch of the experiment tested the hypothesis of whether the density of antlions would have an impact on pit size. We hypothesized that, in situations of greater density, the antlions would all build smaller pits, taking into account the space available and the maintenance costs associated with being near enough to neighbors as to receive flung sand, as well as maximizing the distance between themselves, creating a more uniform distribution. Furthermore, it was hypothesized that fewer pits would be created in order to reduce the stress of high-density situations (Day, 2000; Matsura, 1989).

## METHODS

### *Disturbance and Energy Budget*

Eighty antlions (*Myrmeleon immaculatus*) were gathered from Pine Point at the University of Michigan Biological Station in Pellston, Michigan (N45° 34' 6.53", W84° 41' 9.16"). Antlions were divided into four different treatment groups: one group was left undisturbed; one was disturbed once during the course of the experiment; one was disturbed twice; and the last group was disturbed every other day. Each treatment group contained twenty antlions distributed into two weight classes. Each treatment level had 10 antlions between the weights of 20-40mg and 10 antlions between the weights of 40-60mg. The antlions were housed in deli dishes filled with the second-smallest fraction of sand from a beach on Douglas Lake, the homogeneity of sand throughout the experiment being due to the fact that substrate had an effect on pit size (Roberts, 2007), to within half an inch from the top. Once weighed and transported to their new homes, the antlions were allowed to build a pit for 48 hours. Once their pits were

established, the average diameter was measured, using calipers to measure the length of the longest axis and then the one directly perpendicular to it. One day prior to the experiment, all individuals were fed approximately same-sized ants of the same species (as all ants of the same species show the same caloric value (Griffiths, 1977)), one ant per antlion.

The experiment lasted for a week and antlions were disturbed according to the treatment schedule. Disturbance was defined by filling in the pits to the top with a spoon of sand from the home beach of Pine Point. At the end of the experiment, average pit diameter was measured and the antlions were weighed, and then returned to the wild.

Parameters for analysis included the initial and final weights and the initial and final pit diameter. These data were analyzed using the SPSS interface.

Due to the results of the first portion of the experiment, a follow up experiment was planned. Ninety antlions were collected from Sturgeon Bay in Wilderness State Park, Northern Michigan, in order to test the effects of initial energy budget on pit size. The 90 antlions weighed 40-60mg. The antlions were housed in deli dishes filled with sand from Douglas Lake. Three treatment groups of 30 antlions each existed: one was low energy, another was a control group, and the last was high energy. Within each treatment group, 15 antlions were disturbed every 48 hours using the methodology of the previous experiment, and 15 antlions remained undisturbed. The antlions were arranged in a checkerboard pattern to eliminate any biases. The antlions were allowed to build pits for two days before the beginning of the experiment. Once their pits were established, all antlions were fed one ant each. Weights and average pit diameters were recorded on day three of the six-day pre-experiment.

In order to create disparities in energy budget, the antlions were engaged in a six day pre-experiment. The low energy antlions were placed on a doodle board (a tray with less than a half inch of sand, which forced them to move about in order to find an ideal location to construct a pit) to expend excess energy. The control group was not exercised or fed during the pre-experiment. The high energy group was fed one ant a day for all six days.

The antlions scheduled to be disturbed were disturbed once every 48 hours by filling in the pits with sand. The undisturbed antlions remained undisturbed. This portion of the experiment lasted for one week. At the end of the experiment, weights and average pit diameters were recorded. These data were analyzed, again, using SPSS.

### *Intrinsic and Environmental Factors*

A common garden was established in order to determine whether intrinsic or environmental factors were at work in antlion pit size. The pits of 32 antlions were measured at both Sturgeon Bay (N45° 40' 9.08", W84° 58' 6.40") and Douglas Lake and. These 64 antlions were captured. The antlions all weighed between 20-40mg. Deli tubs with mesh bottoms were arranged approximately 5cm apart on a level surface at Pine Point on Douglas Lake, sunk into the sand to simulate the soil environment as closely as possible. Of the two groups of 32 antlions from both Sturgeon Bay and Douglas Lake, 16 were placed in sifted sand containing granules no larger than 500µm of Douglas Lake sand and the other 16 were placed in the same fraction of Sturgeon Bay sand. The tubs were arranged in a checkerboard pattern (See Figure 3) to control any edge effect, and the experiment was covered with a clear plastic roof to protect the antlions from rain. The antlions were allowed three days to form pits, at which time the antlions were fed one ant and their pits were disturbed by brushing the surface of the sand until the pit was filled completely. After another three days, the pits were measured with calipers, taking the longest diameter and then the diameter perpendicular. These two measurements were averaged to give a

single average diameter value. The antlions were fed and then the entire set up was relocated to Sturgeon Bay. The same feeding, disturbance, and measuring procedures were repeated, and the antlions were released in their home habitat.

Due to the results of the first part of the experiment, a second common garden was set up. In this second experiment, 60 antlion pits were measured and the animals were captured, 30 from Sturgeon Bay and 30 from Douglas Lake. The antlions had weights between 20 and 40mg. All antlions were placed in deli dishes filled with the second smallest fraction of Sturgeon Bay sand and set up in a 10x6 matrix following a checkerboard pattern to eliminate edge effects. The pits were measured every day, and on every third day, the antlions were fed one ant each and disturbed by brushing sand over the pit until it was filled. Measurements continued for a total of 10 days.

### *Density*

In order to understand the natural distribution and population density of antlions, natural densities of antlions at Sturgeon Bay were examined. First, in order to investigate the distribution of pits throughout the sand dunes, a one-meter square frame was thrown randomly 80 times within antlion habitat. The number of pits per square meter was calculated and the data were analyzed in SPSS. Second, 11 one-meter square plots were selected in denser areas, containing between three and fifteen pits per square meter. In these plots, average pit diameter was measured using calipers (taking the longest diameter and the perpendicular diameter) and distance to nearest neighbor, from the center of the focal pit to the outer edge of surrounding pits, was measured using a ruler.

Before the controlled density experiment, the average sand-throwing distance associated with antlion pit construction and maintenance was calculated in order to determine the distributions that would be necessary for the antlions sand flinging to have an effect. Antlions with previously recorded average pit diameters and weights were placed in deli dishes filled with sifted sand from Douglas Lake with granules not exceeding 500 $\mu$ m. The dishes were situated in a pre-cut hole in a large sheet of cardboard so that the cardboard was level with the lip of the deli dish. A sheet of contact paper was placed on top of the cardboard, also with a hole cut to allow for the antlion habitat, with the sticky side facing up to catch any sand that would be flung. The antlions were allowed 48 hours to construct their pits. Of the antlions that took part of the experiment, 11 had constructed their pits near the edge of the cardboard, ensuring that the data for sand-flinging distance was accurate. A wedge was cut of the contact paper extending from the center of the pit to the maximum distance of sand thrown. A compass was used to draw 2cm concentric circle intervals on the contact paper. Sand was scraped from each interval with a micro-spatula and weighed. The proportions of sand thrown into each interval was calculated and then compared to body size and pit diameter.

During the natural density observation, 60 antlions from Sturgeon Bay were gathered. The average pit diameter and nearest neighbor measurements were kept consistent with the corresponding antlion, providing a field and laboratory component to the study. The antlions were all within the weight range of 30-60mg. Data from both the field observation and sand-throwing experiment allowed an estimation of the total space one antlion occupies, including both pit diameter and sand-throwing distance. Each antlion occupied a space of roughly 150 cm<sup>2</sup>.

Four treatments were created within 40x40x10cm wooden boxes, with a total surface area of 16000 cm<sup>2</sup> per box. Each box was filled with the second smallest fraction of sand from Sturgeon Bay. The least-dense treatment contained only four antlions, making the density 0.25

antlions per 100 cm<sup>2</sup>, and a predicted occupation level of 37.5% of the available space. The second treatment contained 8 antlions, with a density of 0.50 antlions per 100 cm<sup>2</sup> and would occupy 75% of the available space. The third treatment housed 16 antlions with a density of 1.00 antlion per 100 cm<sup>2</sup>, requiring 150% of the box surface area. The last treatment, the highest density, held 24 antlions with a density of 1.5 antlions per 100 cm<sup>2</sup> and requiring 225% of the wooden box.

In order to account for confounding variables from intraspecific variation, an experimental set-up was developed, which allowed the tracking of 16 antlions of known body mass and natural pit diameter through each treatment. Using antlions of intermediate weight, between 38 mg and 53 mg, four antlions were assigned to four different groups and the groups were cycled through each treatment level (See Table 1). At the beginning of each trial, the specified number of antlions per treatment was placed in the center of the box on top of smoothed sand. After waiting 48 hours for antlions to complete pit construction, average pit diameter was measured using calipers and nearest neighbor distance was discerned using a ruler. Following the measurements, each antlion was removed and the tracked antlions were moved to their next trial, keeping the non-tracked antlions in the same treatment level throughout the experiment. Due to increased solar radiation on some treatment boxes, the second trial was repeated, as this was a threat to the homogeneity of the environment. The entire experiment, including the repeated second trial, lasted ten days.

Nearest neighbor distances were grouped into 1 cm bins. The data were analyzed using SPSS. Several Poisson distributions were used to ascertain whether or not the nearest neighbor distances showed random distribution. The resulting two-way contingency table was analyzed using a G-test.

## RESULTS

### *Disturbance and Energy Budget*

SPSS was used to analyze the data. Tests of normality were performed on both initial diameters and final diameters in order to determine whether parametric or nonparametric tests would be utilized. The p-values for all diameters were greater than the 0.05 significant difference threshold so parametric tests were used. Due to the nature of the data, a Repeated Measures Analysis of Variance (ANOVA) was performed on average initial and final pit diameters. The pit diameter varied significantly over time ( $F=6.404$ ,  $df=1$ ,  $p=0.014$ ). The time and weight class also displayed a significant difference ( $F=6.317$ ,  $df=1$ ,  $p=0.015$ ). Time and treatment interaction had no significant effect on pit diameter ( $F=0.137$ ,  $df=3$ ,  $p=0.938$ ), and neither did the interaction between time, treatment, and weight class ( $F=1.689$ ,  $df=3$ ,  $p=0.181$ ). Pit size did not change significantly due to disturbance treatment. The test of between-subjects effects showed that, between weight classes, the pit diameter did vary significantly between initial and final, with a larger change in pit diameter from initial to final resulting in the larger weight class ( $F=17.958$ ,  $df=1$ ,  $p<0.0001$ ) (See Figure 4). The hypothesis that disturbance affected energy budget was supported; however, the hypothesis that suggested that the effect on pit size would differ according to the level of disturbance was not supported.

A test of normality was performed on the weight loss data as well. The data were not normal, due to the presence of two expected outliers. Two antlions in the data set gained weight between the beginning and the end of the experiment. The experimental parameters established would not allow for a gain in weight, as it suggests that the antlion discovered a source of food

not accounted for in the experiment. It is possible that a food item found its way into the antlion's dish. As such, the two figures that indicated an increase in antlion weight were discarded. The data were still not-normal ( $p=0.005$ ), so a nonparametric Kruskal-Wallis test was performed in order to test for significance. The weight loss data were significantly different (Chi-Square=22.339,  $df=3$ ,  $p<0.0001$ ). Post-Hoc Bonferroni and Tamhane tests were completed to conclude which weight losses were statistically significantly different. The group that was not disturbed differed significantly from the groups disturbed every other day (Bonferroni  $p=0.01$ , Tamhane  $p=0.004$ ), every three days (Bonferroni  $p<0.0001$ , Tamhane  $p<0.0001$ ), and only once during the course of the experiment (Bonferroni  $p=0.004$ , Tamhane  $p=0.007$ ) (See Figure 5). Weight loss between weight classes was also tested for significance using a Kruskal Wallis test (due to the abnormal distribution of the weight loss data determined previously). The differences in weight loss between weight classes were not statistically significantly different (Chi-Square=2.255,  $df=1$ ,  $p=0.133$ ). The hypothesis that disturbed antlions would lose more weight due to the increased energetic stress of maintaining a frequently disturbed pit was not supported.

SPSS was also used to analyze the data from part two of the experiment. Tests of normality were run on the weight loss and average pit diameter data. Both data sets revealed abnormal distributions, therefore the analyses proceeded using nonparametric tests. A Kruskal-Wallis test revealed that energy level did have a significant effect on weight loss (Chi-square=19.373,  $df=2$ ,  $p<0.0001$ ). A post-hoc Bonferroni test revealed that the low energy group differed significantly from the high-energy group ( $p<0.0001$ ). The control group also differed significantly from the high-energy group ( $p=0.004$ ) (See Figure 6). A Kruskal-Wallis test was performed to examine the effect of disturbance group on weights, but no significant effect was detected (See Figure 7). The hypothesis that stated that antlions with lower energy budgets would build smaller pits was not supported.

The data for average pit diameter were analyzed. Disturbance level did have a significant effect on the change in average pit diameter, with the undisturbed groups having larger pits than the disturbed groups (Chi-square=32.782,  $df=1$ ,  $p<0.0001$ ) (See Figure 8). Once again, our hypothesis regarding the decrease of pit size with increased disturbance was supported. A Kruskal-Wallis test on the relationship between energy level and pit size yielded no significant result.

### *Intrinsic and Environmental Factors*

A three-way ANOVA was performed, with the dependent variable being average pit diameter, the independent variables as site, sand, and antlion origin, and the covariate was weight. The results showed that Douglas Lake antlions made significantly different pits from the Sturgeon Bay antlions ( $F=9.580$ ,  $df=1$ ,  $p=0.003$ ). Sturgeon Bay antlions made significantly larger pits than the Douglas Lake antlions (See Figure 8). This supports the hypothesis that intrinsic factors are at work in antlion pit size, because environment had the opposite of the expected effect, with only the antlion origin was at work. Weight also showed a significant result, with larger antlions producing larger pits ( $F=5.169$ ,  $df=1$ ,  $p=0.028$ ) (See Figure 9). A summary of the findings of the ANOVA is presented in Table 2.

Several Mann-Whitney tests were performed to determine if pit diameters differed per day. During the second experiment, pit diameters were significantly different on day 0 ( $Z=-4.932$ ,  $p<0.001$ ) and day 10 ( $Z=-2.428$ ,  $p=0.015$ ) between Sturgeon Bay and Douglas Lake antlions (See Figure 10). Days 1 through 9 did not show statistically significant differences in pit size between Sturgeon Bay and Douglas Lake antlions (See Table 3). This does not support the



intrinsic factor hypothesis because, when placed in similar environments, the antlions built the same size pits regardless of origin.

### *Density*

Of the 80 randomly-selected, one-meter square plots, 45 contained no antlions. These sample plots were not used in the analysis of natural density due to the fact that these plots may represent unsuitable antlion habitat (and the experiment focused on antlion density within suitable habitat). A Chi-Square test was used to analyze the data. A Poisson distribution was used to calculate the expected pits per square meter, given a random distribution. The antlion pit distribution was not random: Significantly higher frequencies occurred at 1 pit per square meter, 6 pits per square meter, and 11 pits per square meter; significantly lower frequencies than expected occurred at 3 pits to 5 pits per square meter ( $\chi^2=107.0227$ ,  $df=7$ ,  $p<0.001$ ) (See Figure 11). A regression analysis of nearest neighbor distance and its effect on pit diameter showed a slight positive correlation ( $F_{1,48}=5.36$ ,  $p=0.0251$ ,  $R^2=0.08$ ) (See Figure 12).

Ten of the antlions threw sand into the 2 and 4 cm intervals. The proportion of sand thrown up to 4 cm accounted for between 59% and 100% of total sand thrown, containing an average of 81% of total sand thrown. Nine of the antlions threw an average of 18% of total sand displaced into the 6 cm interval, and three antlions threw an average of 6.6% of total sand into the 8 cm interval (See Figure 13).

A two-way ANOVA was performed to compare the effect of density on pit diameter. The results included a significant treatment effect, suggesting that density significantly decreased pit size ( $F=4.52$ ,  $df=3$ ,  $p=0.009$ ). A post-hoc Tukey test revealed that the high-density treatment, treatment four, differed significantly from treatments two and three ( $MS=38.618$ ,  $df=35$ ,  $p=0.0227$ ;  $MS=38.618$ ,  $df=35$ ,  $p=0.0225$  respectively). The high-density treatment did not differ significantly from the low-density treatment ( $p=0.0682$ ), and treatments one, two, and three did not differ from each other ( $p=0.947$ ,  $p=1.00$ ,  $p=0.945$  respectively) (See Figure 14). A significant group effect and a significant treatment-group interaction were also detected ( $F=2.88$ ,  $df=3$ ,  $p=0.0492$ ;  $F=3.597$ ,  $df=9$ ,  $p=0.0029$ ). Antlion's pit building varied between groups and the response to treatment level depended on the inter-group variation.

A two-way ANOVA was completed to test the effect of density on the average nearest neighbor distance. The treatment level had a significant effect in decreasing the distance between pits ( $F=14.985$ ,  $df=3$ ,  $p<0.001$ ). A post-hoc Tukey test revealed that treatments one and two did not differ significantly ( $p=0.440$ ), but that treatment one differed from treatments 3 and 4 ( $MS=6.763$ ,  $df=35$ ,  $p<0.001$ ;  $MS=6.763$ ,  $df=35$ ,  $p<0.001$  respectively). Treatment two was significantly different from treatments three and four ( $MS=6.763$ ,  $df=35$ ,  $p<0.001$ ;  $MS=6.763$ ,  $df=35$ ,  $p=0.003$ ). Treatments three and four did not display significantly different nearest-neighbor distances ( $p=0.958$ ). The average nearest neighbor distances for each treatment were compared to a Poisson distribution to determine whether or not nearest neighbor distances became more uniform as density increased. The second, third, and fourth treatments did not differ significantly from a Poisson random distribution ( $\chi^2=16.242$ ,  $df=17$ ,  $p=0.5067$ ;  $\chi^2=7.355$ ,  $df=14$ ,  $p=0.90211$ ;  $\chi^2=20.608$ ,  $df=14$ ,  $p=0.1121$  respectively).

According to a Chi-Squared Goodness of Fit test, treatment one had significantly different average nearest neighbor distances than predicted by the Poisson distribution ( $\chi^2=32.463$ ,  $df=17$ ,  $p=0.0132$ ) (See Figure 15), with much higher than expected nearest neighbor distances in the 16 to 19 cm range, suggesting that in low-density settings, antlions attempt to maximize the distance between competing neighbors and do not randomly choose pit locations.

Treatment two nearest neighbor distances were more uniform than treatment 3 (See Figures 16 and 17) but may be due to an unrepresentative sample size ( $n=24$ ). The average nearest neighbor distances from treatment four were more uniform than treatment three (See Figures 17 and 18).

The number of pits built at different treatment levels was compared to determine if density affected the probability of antlion pit building. The number of pits built was significantly different between treatments, according to a G-test ( $G=8.91$ ,  $df=3$ ,  $p<0.05$ ). Increased pit density did not decrease the likelihood of pit construction. A trend is visible with the antlions in the low-density situations building proportionately fewer pits than the antlions in high-density situations (See Figure 20).

## DISCUSSION

### *Disturbance and Energy Budget*

In the first part of the experiment, the larger weight class of antlions lost more weight (See Figure 4), perhaps implying an energetic effect on maintaining pit size; however, pit size did not rely on energetic budget in the second part of the experiment, only the disturbance level did (See Figure 7). Though the set-up for the energy budget experiment succeeded in creating a high-energy group that was statistically different from both the low-energy and control group (See Figure 6), the experiment failed to establish and maintain a distinct energetic difference between the low-energy and control groups. This may have affected the results; perhaps the low-energy groups simply did not reach a level of exhaustion at which they would be forced to reduce pit size in order to save their energy. Antlions are able to resist starvation for long periods of time. *M. immaculatus* can survive for up to three months without a meal and only start showing effects of starvation after day 20 (Heinrich, 1984). Also, in an experiment involving a different species of antlion (*M. bore*), the larvae responded differently to starvation (Matsura, 1987). Instead of changing the position of their sites, they would flip more sand out of the bottom of their pit, creating a larger pit. Therefore, the pit size increased with the level of starvation, reaching the target size only when sand-flinging capacity was stifled by starvation-induced weakness. This also supports the hypothesis that antlions show a mechanism for withstanding starvation over long periods of time, and it has been suggested that antlions reduce their metabolic rate like net-spinning spiders (Matsura, 1987).

The size of the ant fed to the antlions probably did not have an effect either way in creating the energetic budget, considering that Griffiths (1977) showed that the caloric value of insects is not tied directly to body size. Because the ants provided to the antlions were all of the same species, the caloric value was probably about the same.

In the second part of the experiment, energetic budget did not have an effect on pit size, and all of the antlions lost weight over time (suggesting the energetic budget involved with pit construction). Scientists have observed two contradictory reactions to high levels of feeding in antlion larvae. The first response sees antlions reducing their pit diameter when well-fed, indicating that their pit size is sufficient to catch prey (Arnett, 2001). A second response has antlions building a larger pit due to available energy stores and the prospects of continued prey capture (Griffiths, 1986). The antlions may have been willing to sacrifice the energy involved in pit building due to the associated advantages. Building pits (and therefore rebuilding pits following disturbance) is advantageous for antlions because they are free from predation (Scharf, 2006). Predators of antlions include small reptiles (Simon, 1989), birds (Hauber, 1999), as well as group-foraging ants and cannibalistic antlions (Lucas, 1986). Also, because the antlions are in

the larval form, the larvae are more likely to optimize their food intake rather than minimizing time (Griffiths, 1980) because this optimization of food intake is required in order to reach the next instar, regardless of time.

### *Intrinsic and Environmental Factors*

Sturgeon Bay antlions built significantly larger pits than Douglas Lake antlions regardless of in which site they were planted except in the lab. (See Figure 8). This suggests that environment (including soil dynamics and openness to disturbance through the elements, because the sand granule size was controlled for by using the same fraction of sand from both Douglas Lake and Sturgeon Bay) has little to no effect on pit size, suggesting that intrinsic factors, either genetics, learning, or physiological conditioning could affect the pit size.

Genetics may play an important role in the determination of pit size. Both genetic and environmental factors combine to control antlion body size, which has been previously positively correlated with pit size (Roberts, 2007).

In the second portion of the experiment, the pits built by both Sturgeon Bay and Douglas Lake antlions in the laboratory setting converged on a point, which seems to contradict a genetic factor to pit size, as the antlions were able to take note of their surroundings and change their pit size accordingly (See Figure 10). When placed in similar situations, constrained by the deli dish, the antlions built approximately the same size pit. This suggests, contrary to the previous experiment, that environment plays a more major role in pit construction.

A study by Guillette (2009) suggested an associative learning to occur with the construction of antlion pits. Antlions that had the benefit of learning through the presentation of prey constructed larger pits than those who did not have the benefit of learning. In the same experiment, antlions that learned to recognize the appearance of prey and clear their mandibles of sand had more effective capture and therefore could lessen the time associated with a given meal. This learning is beneficial to the organism because faster larval growth leads to pupation sooner, thereby completing the antlion's goal of reproducing while reducing the time spent open to predation and disturbance by other factors. The antlions who exhibited learning had a 20% greater growth rate and reached the next instar stage 7% faster than the control group. All in all, learning as a means to increase prey capture by the sit-and-wait predator is beneficial to the end goal of pupation followed by reproduction. Antlions who are able to learn are more fit than other antlions, visible in their accelerated progression through the instars.

Due to the inability of scientists to raise antlions from egg to adult in captivity, genetic analyses have not been completed. In further experimentation, a common garden should be established with close relatives and unrelated larvae in order to determine the true genetic factors involved in pit construction.

### *Density*

The natural densities of antlion pits at Sturgeon Bay differed significantly from the Poisson random distribution. This suggests that the distribution of antlions is not random, that it depends upon outside factors. Studies have concluded that several factors may account for this organized distribution, including avoidance of high temperatures and the availability of shade (Heinrich, 1984).

Density did significantly change pit size (See Figure 14). The antlions in treatment four built significantly larger pits than treatment groups two and three. Previous studies have drawn various conclusions regarding the density aspect of pit-building. In studies where pit densities

were as high as 12.8 antlions per 100 cm<sup>2</sup>, a significant result was extracted, leading to the conclusion that at higher densities, pit size decreased (Day, 2000). However, in a similar study with a density of 5 antlions per 100 cm<sup>2</sup>, no significant decrease in pit size was observed (McClure, 1976). Perhaps a study that incorporated a wider range of densities, from very sparse distributions to very dense distributions, would provide better data from which to conclude the effect of density on pit size. It has also been suggested that antlion behavior may be species specific (Rosenberg, 1987), and therefore it is not possible to compare this work (using *Myrmeleon immaculatus*) with the work by Day, who experimented with *Myrmeleon acer*.

A few sources of error may have contributed to the results of pit diameter. The existence of a group effect in the tracked individuals suggests that the four groups of tracked individuals were not randomly assorted; that is, pre-existing differences between the groups of tracked individuals were observed, and the significant interaction between treatment and group suggest that the different groups responded differently to varying density situations. This may decrease the validity of some conclusions drawn from the laboratory density treatments, as the groups, once determined, did not assort. It is therefore possible that the lack of random assortment of the groups could lead to unforeseen bias in the rest of the experiment.

Density treatment had a significant effect on nearest neighbor distances (See Figure 19). Treatment one, the low-density treatment, differed significantly from treatments three and four, the middle-density treatments, as did treatment two. As a general trend, nearest neighbor distances decreased as the density of antlions increased, and the distributions of nearest neighbor distance became more uniform from low density to high density. The same trend was witnessed by McClure (1976). At the highest experimental densities, the larval distribution was extremely regular and it approached the maximum spatial uniformity. At lower densities, this uniformity was not witnessed.

Antlions at higher densities had a significantly higher number of pits built (See Figure 20). Preceding studies observed an increasing number of antlions that did not build pits with increased situations of densities (Matsura, 1989; Griffiths, 1991; Day, 2000). In one instance, pit building decreased by 26% (McClure, 1976). This suggests that, instead of decreasing pit size overall, some individuals were excluded from pit building. However, in this experiment, the antlions continued to build pits despite the density situation. Again, this may be due to the differences in behavior from one antlion species to the next, or perhaps the highest density situation was not dense enough to display this decrease in pit-building.

Antlions should be responsive to density. Interference competition occurs when one sit-and-wait predator interrupts the flow of prey to another sit-and-wait predator, as when one antlion builds a pit upstream from another and therefore receives all the benefits (Linton, 1991). Antlions, as energy maximizers in terms of energetic strategy, would choose to minimize obstructions on the pit periphery in order to gain the most energy as necessary, leading to pupation. Antlions in high-density situations also deal with sand thrown by their neighbors and are vulnerable to cannibalism.

A complex interplay between disturbance, intrinsic factors, and density determine the size of antlion pits. Antlions that experience high levels of disturbance build smaller pits, and pit size can span different environments. If existing in high-density situations, antlions will also build larger pits. The pit-building capabilities of antlion larvae are imperative for their survival: without a pit, an antlion has no prey and no way of reaching the next instar stage. The abiotic

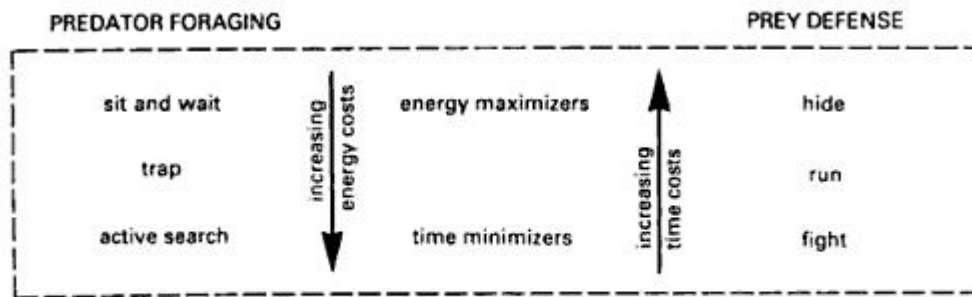
factors contributing to antlion pit diameter have been widely studied; the biotic factors, including genetics, require further investigation.

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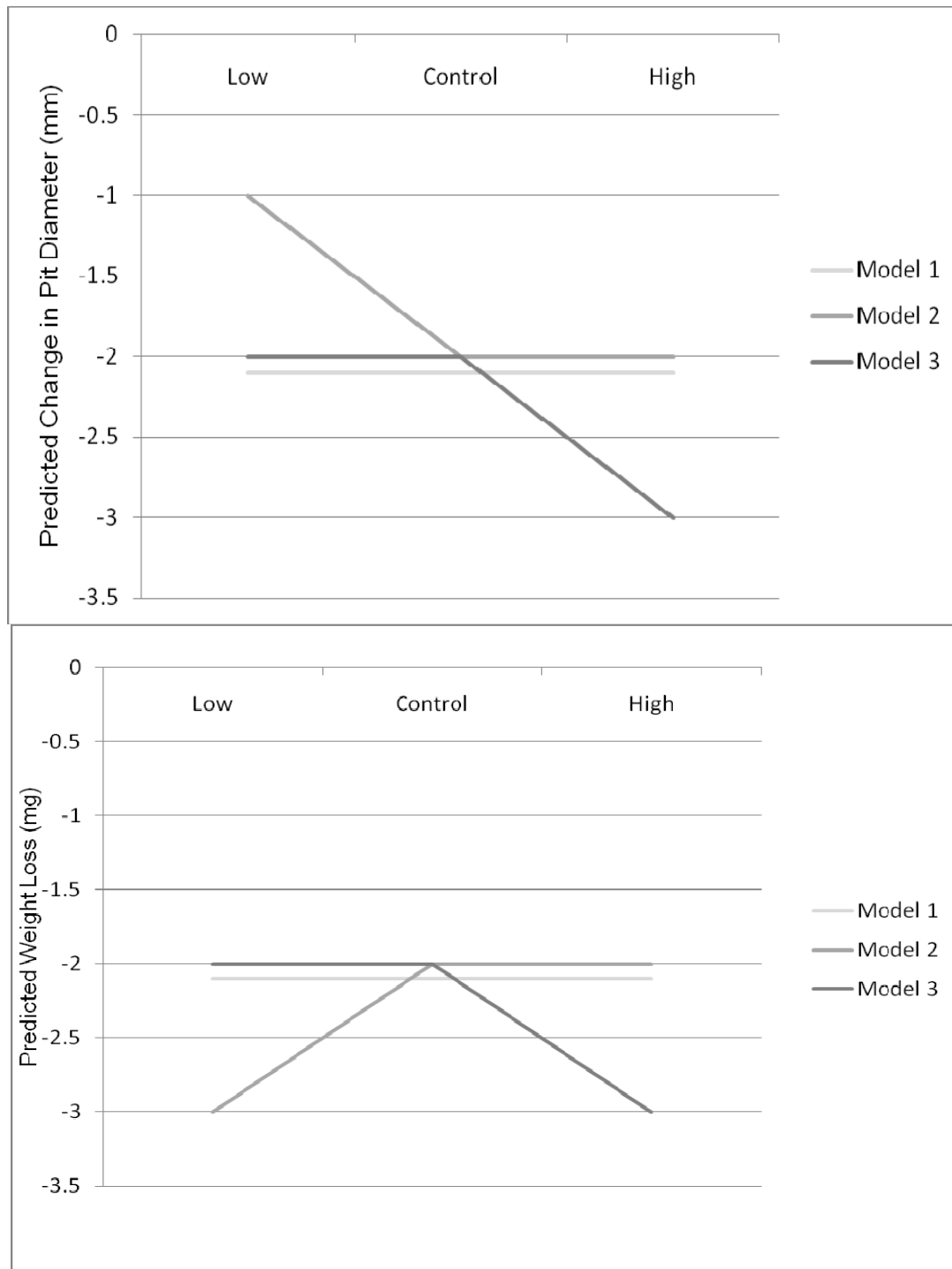
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## FIGURES



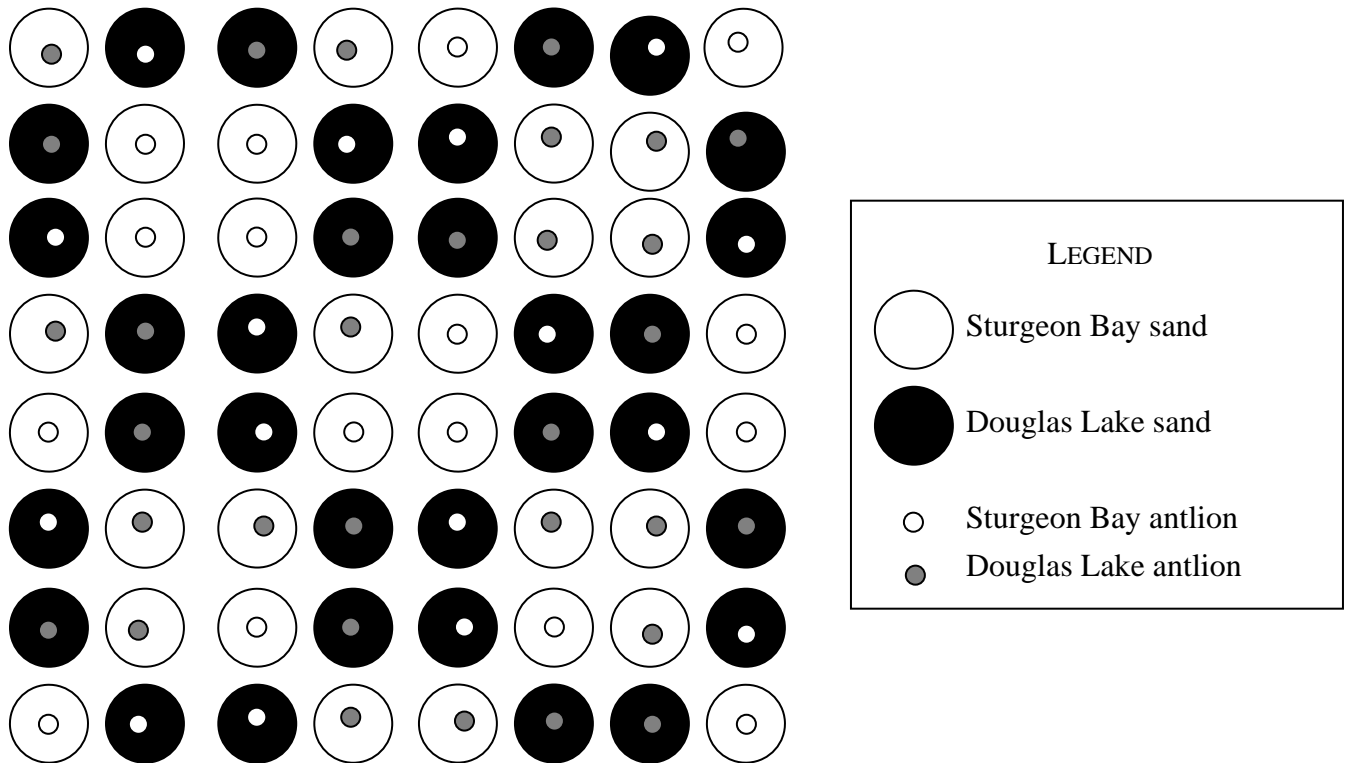
**Fig. 13.1** The basic spectrum of predator foraging and prey defense strategies, varying according to costs and benefits in both time and energy. (After Malcolm 1990.)

**Figure 1: Foraging strategies and associated energetic costs for insects.** The sit-and-wait model of predation is an energy maximizing and time-intensive strategy for food acquisition. (Gullan, 2005).



**Figure 2: Model predictions of effects of disturbance on average pit diameter and weight loss (energetic budget).** Three models present three possible predicted outcomes of the disturbance and energetic budget experiment. Values on Y-axis are arbitrary.

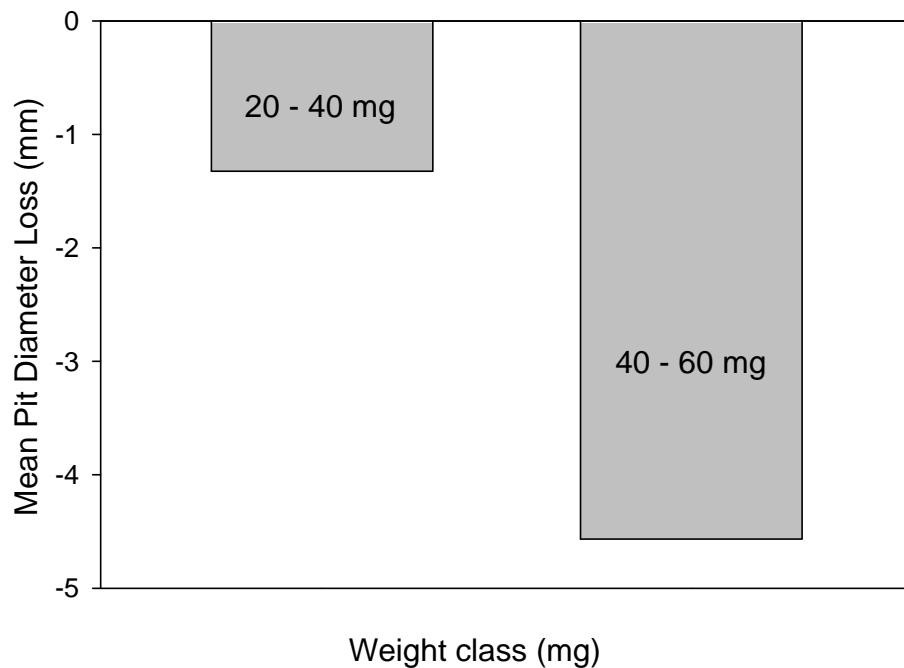




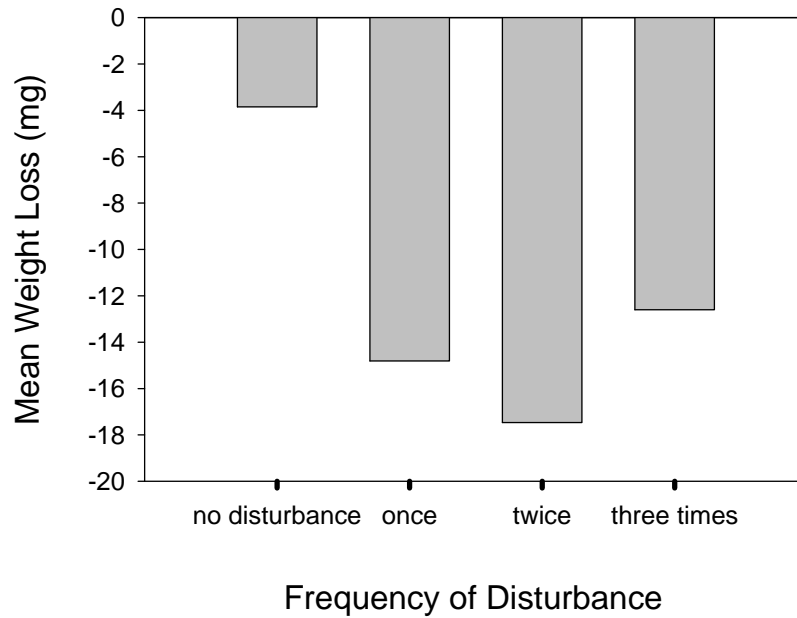
**Figure 3: The checkerboard pattern instituted for the common garden experiment.** Of the 32 Sturgeon Bay antlions, 16 were placed in Douglas Lake sand and 16 remained in Sturgeon Bay sand. Of the 32 Douglas Lake antlions, 16 remained in Douglas Lake sand and 16 were placed in Sturgeon Bay sand.

	Trial #1	Trial #2	Trial #3	Trial #4
Treatment #1 0.25 antlions/ 100cm <sup>2</sup>	Group #1	Group #4	Group #3	Group #2
Treatment #2 0.5 antlions/ 100cm <sup>2</sup>	Group #2	Group #1	Group #4	Group #3
Treatment #3 0.1 antlions/ 100cm <sup>2</sup>	Group #3	Group #2	Group #1	Group #4
Treatment #4 1.5 antlions/ 100cm <sup>2</sup>	Group #4	Group #3	Group #2	Group #1

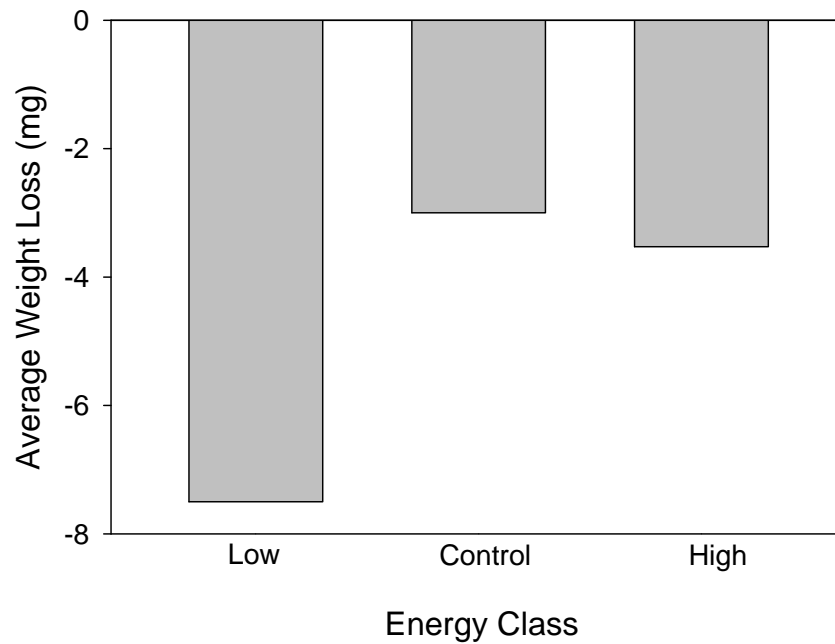
**Table 1: Tracked antlion experimental set-up for density experiments.** In order to reduce intraspecific variation, 16 antlions with previously recorded natural pit diameter and weights were cycled through the treatments.



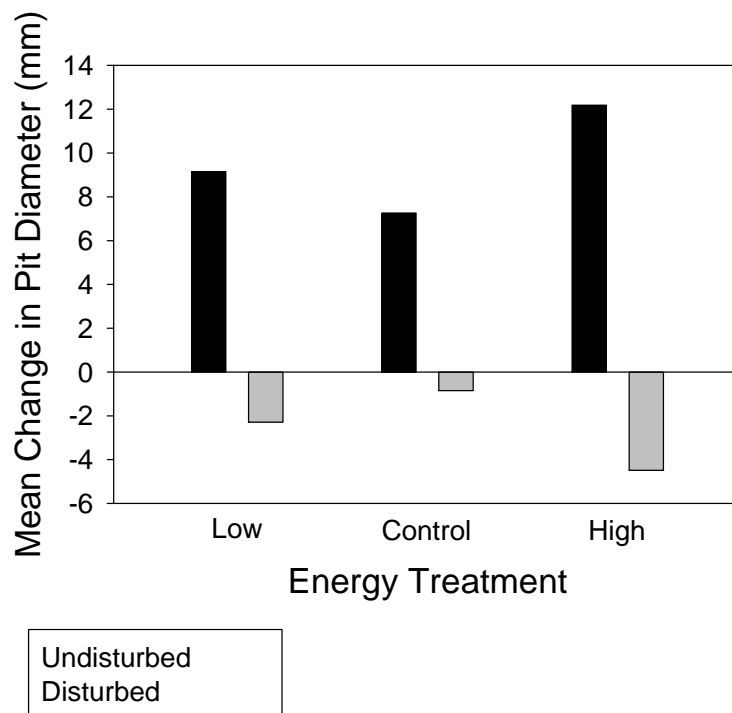
**Figure 4: The interaction between weight class and mean pit diameter loss.** A significant difference in mean pit diameter loss dependent upon weight class was observed ( $F=17.958$ ,  $df=1$ ,  $p<0.0001$ ). The larger weight class's pits lost significantly more diameter than the smaller weight class.



**Figure 5: The effect of disturbance on weight loss.** The group that was not disturbed at all differed significantly from the group disturbed once (Bonferroni  $p=0.004$ , Tamhane  $p=0.007$ ), the group disturbed every three days (Bonferroni  $p<0.0001$ , Tamhane  $p<0.0001$ ), and the group disturbed every other day (Bonferroni  $p=0.01$ , Tamhane  $p=0.004$ ). This suggests that a statistical difference exists between the amount of energy expended by disturbed and undisturbed antlions, but does not imply differences within the levels of disturbance treatment.



**Figure 6: The effect of energy treatment on pre-experiment weight loss.** The pre-treatment did have a significant effect with creating the different energy groups. The low energy class lost more than the control and the high-energy group, though the high-energy group failed to continue gaining weight throughout the experiment.



**Figure 8: The effects of disturbance and energy treatment on mean pit diameter.**

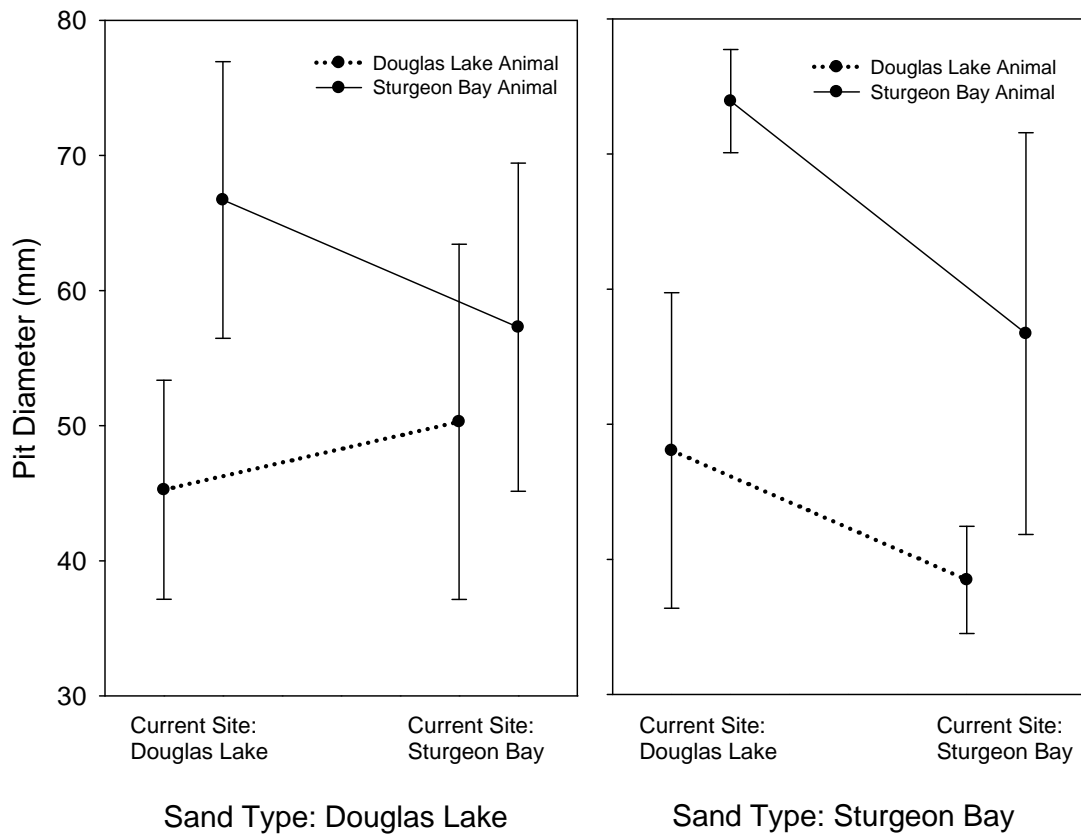


**Figure 7: The effect of disturbance on pit diameter.** Antlion pit diameter decreased significantly dependent on the level of disturbance (Chi-square=32.782, df=1,  $p<0.001$ ).

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7901.829 <sup>a</sup>	9	877.981	5.287	.000
Intercept	1918.172	1	1918.172	11.551	.001
Weight	858.390	1	858.390	5.169	.028
OrigPIT	12.012	1	12.012	.072	.789
Site	386.788	1	386.788	2.329	.134
SandType	21.966	1	21.966	.132	.718
AntlionType	1590.899	1	1590.899	9.580	.003
Site * SandType	194.270	1	194.270	1.170	.285
Site * AntlionType	1.215	1	1.215	.007	.932
SandType * AntlionType	27.547	1	27.547	.166	.686
Site * SandType * AntlionType	77.292	1	77.292	.465	.499
Error	7140.496	43	166.058		
Total	175405.828	53			
Corrected Total	15042.325	52			

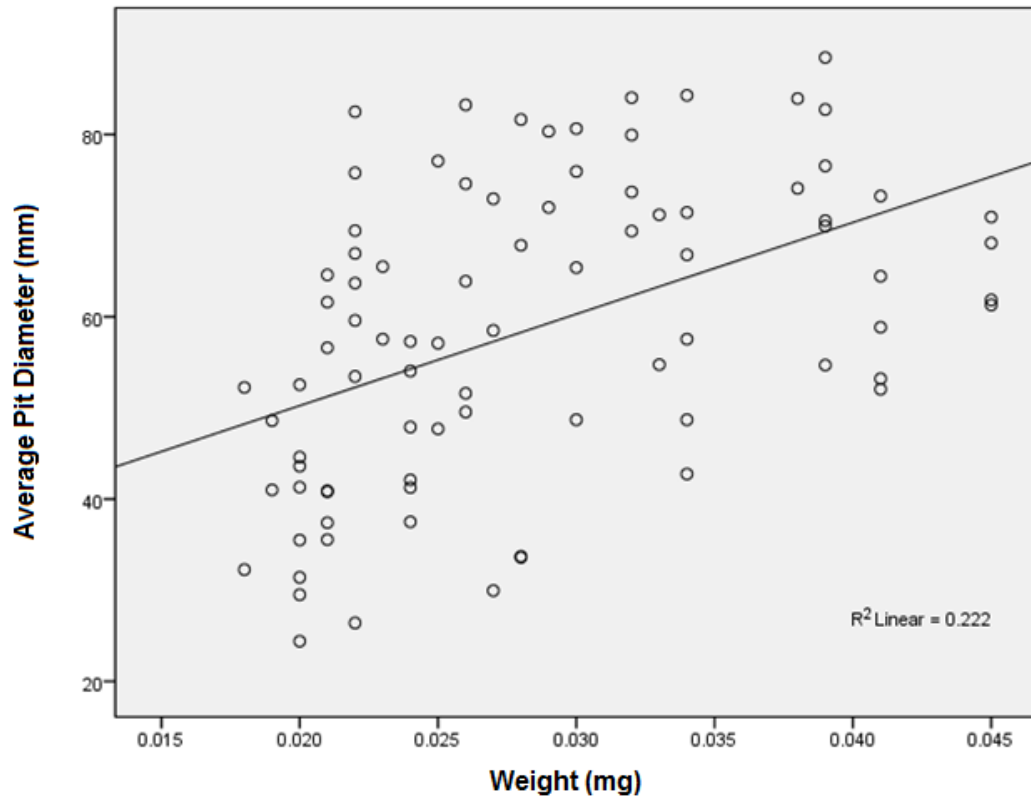
**Table 2: A summary of the univariate tests performed on common garden learning data.**

Antlions from Sturgeon Bay made significantly different pits from Douglas Lake antlions ( $F=9.580$ ,  $df=1$ ,  $p=0.003$ ). Weight was also statistically significant, with larger antlions creating larger pits ( $F=5.169$ ,  $df=1$ ,  $p=0.028$ ).



**Figure 8: Pit size differences between antlions from different sites.** Univariate testing revealed that antlions from Sturgeon Bay made significantly larger pits than Douglas Lake antlions ( $F=9.580$ ,  $df=1$ ,  $p=0.003$ ).

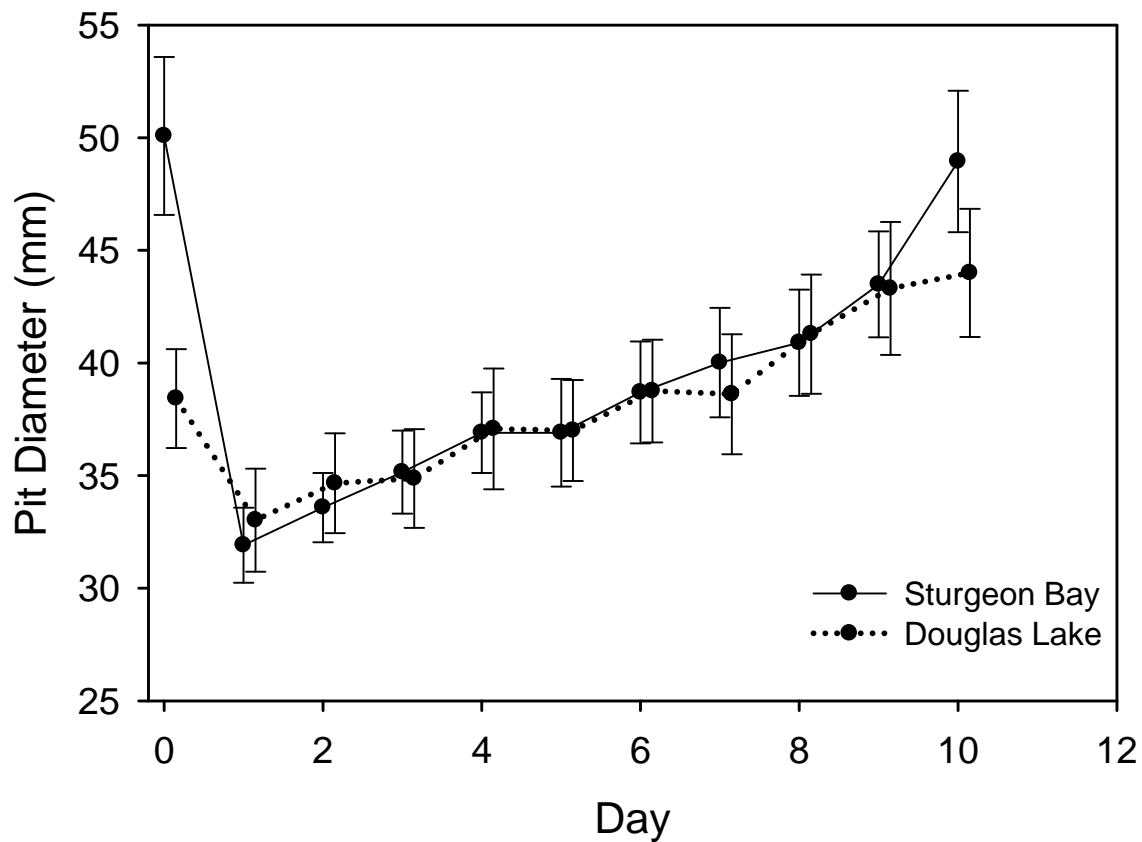




**Figure 9: Regression analysis of the relationship between antlion weight and average pit diameter.** A positive correlation exists between antlion weight and average pit diameter. Univariate testing revealed a significant difference between larger and smaller antlion pit size ( $F=5.169$ ,  $df=1$ ,  $p=0.028$ ).

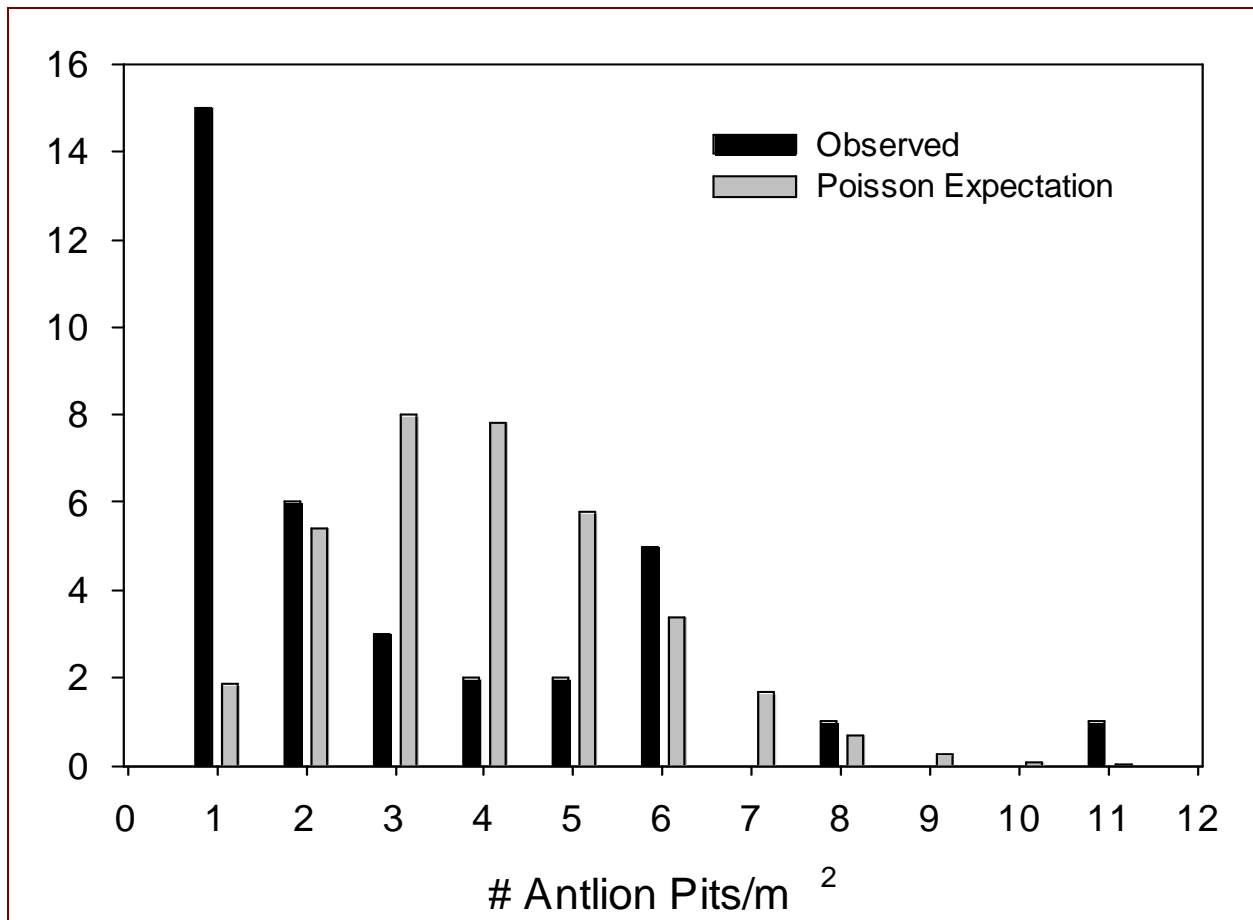
Day	Z	p-value
1	-1.365	0.172
2	-0.526	0.599
3	-0.019	0.985
4	-0.053	0.958
5	-0.586	0.558
6	-0.086	0.932
7	-1.004	0.315
8	-0.079	0.937
9	-0.015	0.988

**Table 3: Statistical results of univariate testing between antlion pit size in learning experiment.** In the laboratory, pit size was significantly different on days 0 ( $Z=-4.932$ ,  $p<0.001$ ) and 10 ( $Z=-2.428$ ,  $p=0.015$ ). The antlion pit sizes on days between did not have significant differences.

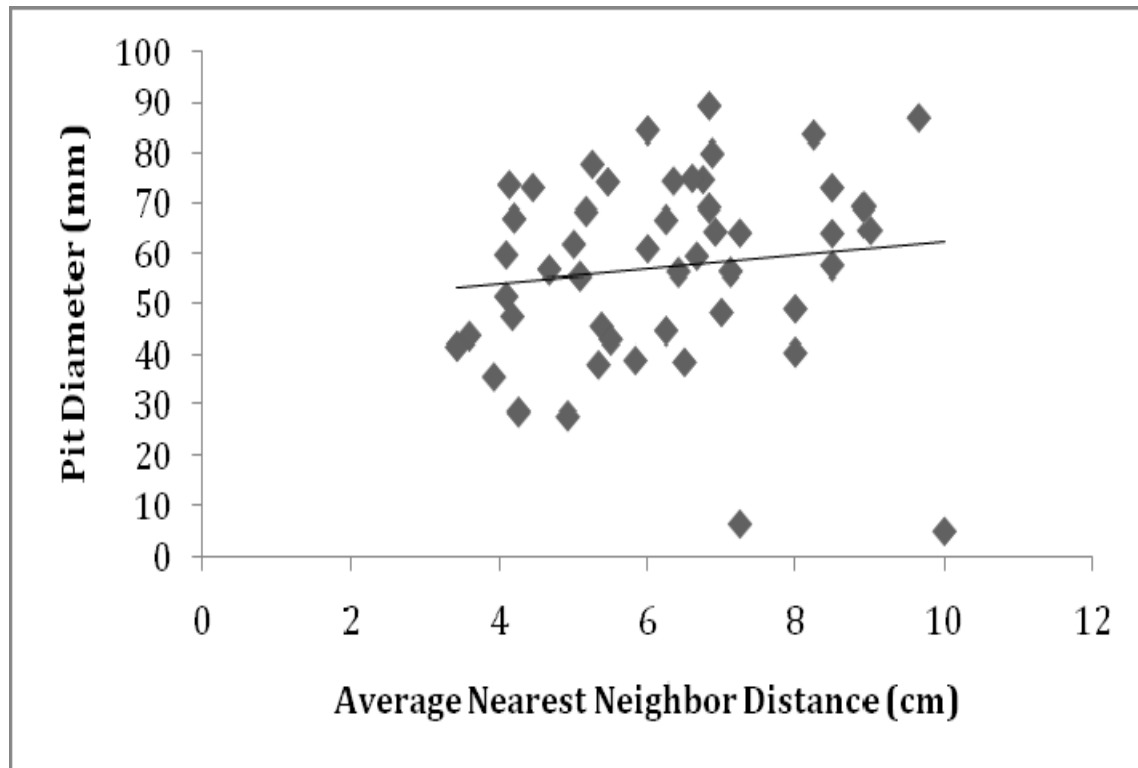


**Figure 10: Average pit diameter convergence in Sturgeon Bay and Douglas Lake antlions.**

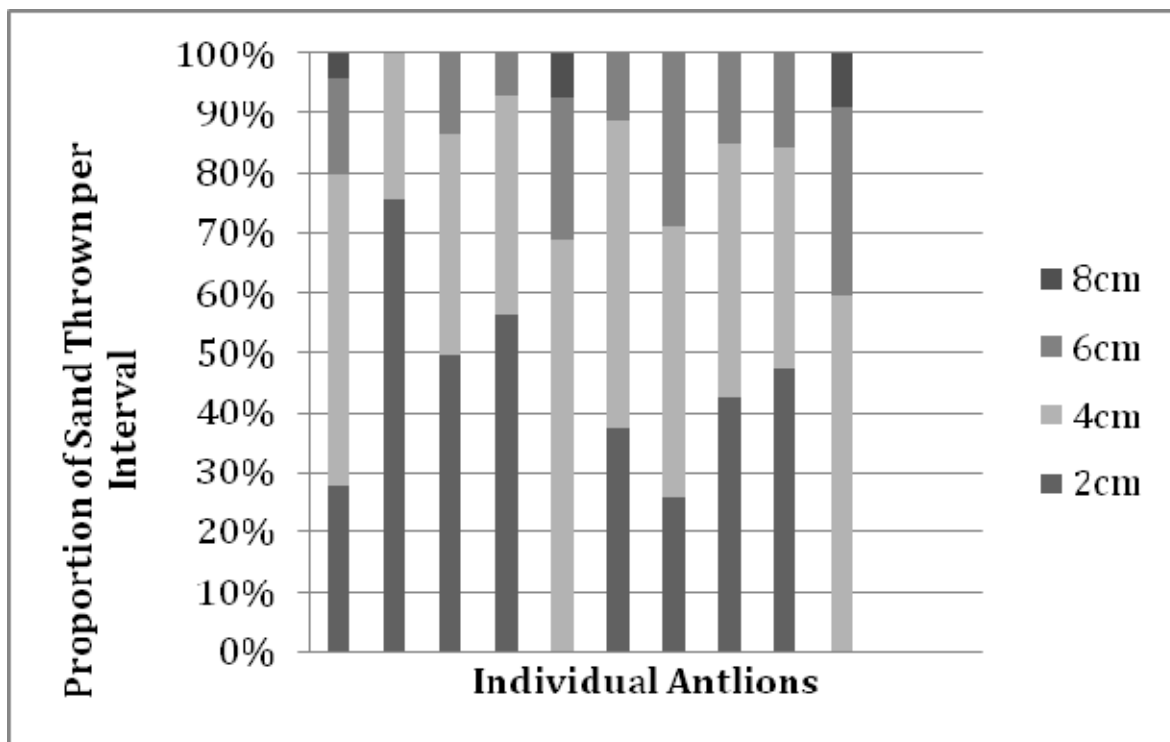
Disturbances occurred on days 0,3,6, and 9. Average pit diameters were statistically significantly different on day 0 ( $Z=4.932$ ,  $p<0.001$ ) and day 10 ( $Z=2.428$ ,  $p=0.015$ ). The results of days 1 through 9 were insignificant.



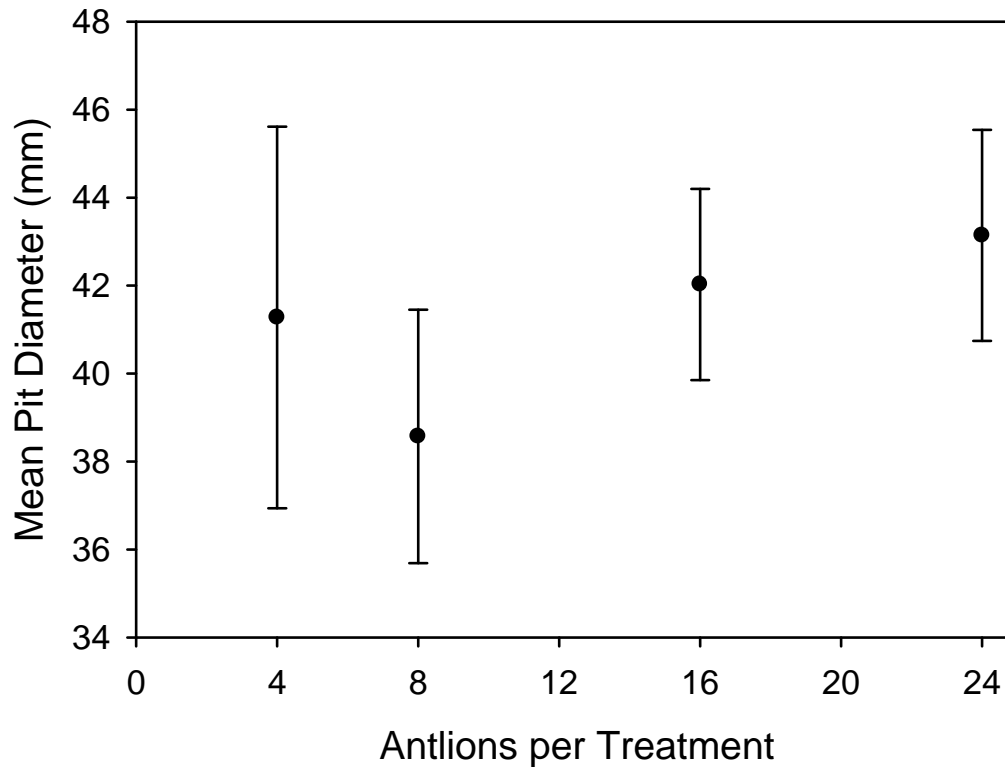
**Figure 11: Poisson distribution of antlion pit density.** The natural distribution of pits within one square meter was not random. Significantly higher frequencies occurred at 1 pit per square meter, 6 pits per square meter, and 11 pits per square meter. Significantly lower frequencies than expected occurred at 3 pits to 5 pits per square meter ( $\chi^2=107.0227$ ,  $df=7$ ,  $p<0.001$ ).



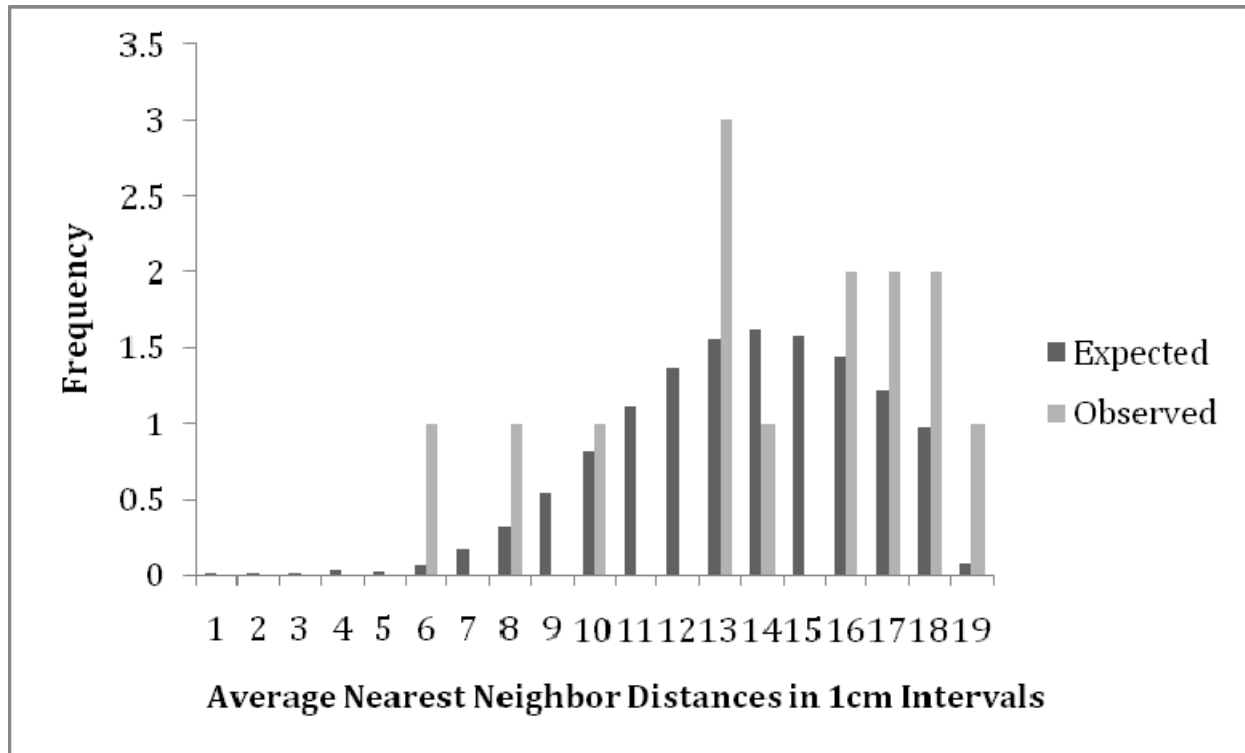
**Figure 12: Regression analysis of average nearest neighbor distance and pit diameter.** A regression analysis of nearest neighbor distance and its effect on pit diameter showed a slight positive correlation between the two variables ( $F_{(1, 48)}=5.36$ ,  $p=0.0251$ ,  $R^2=0.08$ ).



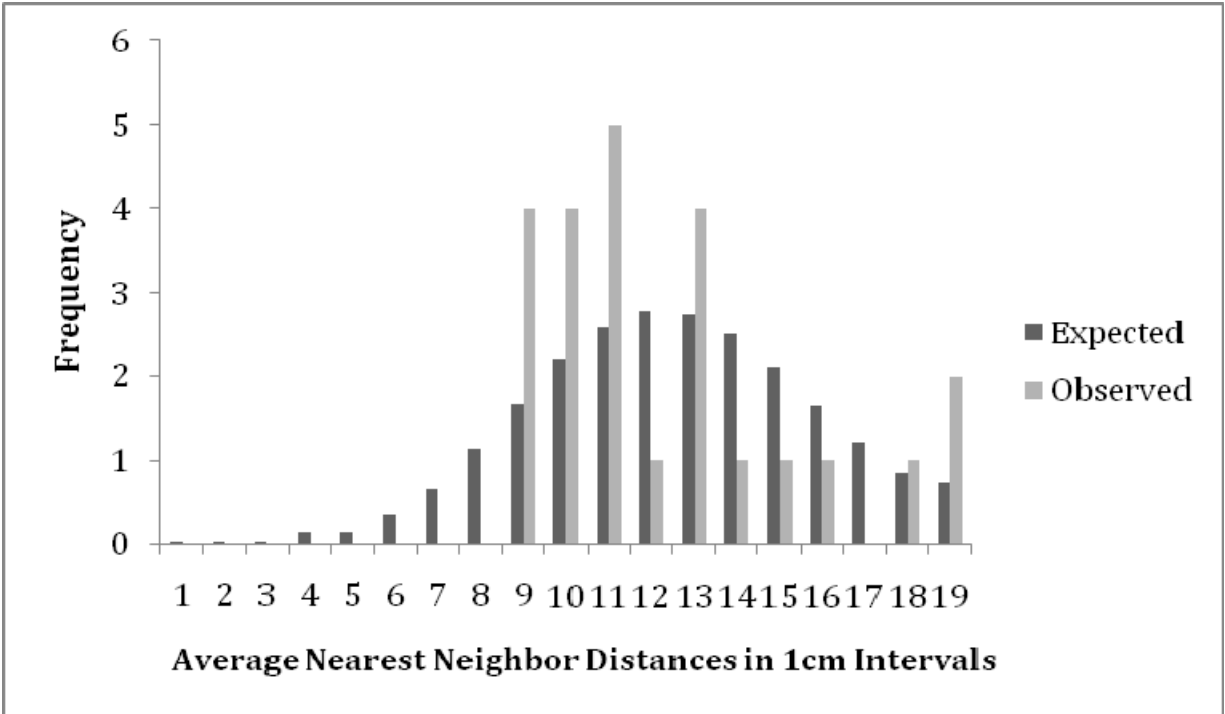
**Figure 13: Proportions of sand flung by interval.** The proportion of sand thrown up to 4 cm accounted for 59-100% of the total sand displaced and contained an average of 81% of the total sand. An average of 18% of total sand was thrown into the 6 cm interval by nine antlions, and three antlions threw an average of 6.6% of total sand into the 8 cm interval.



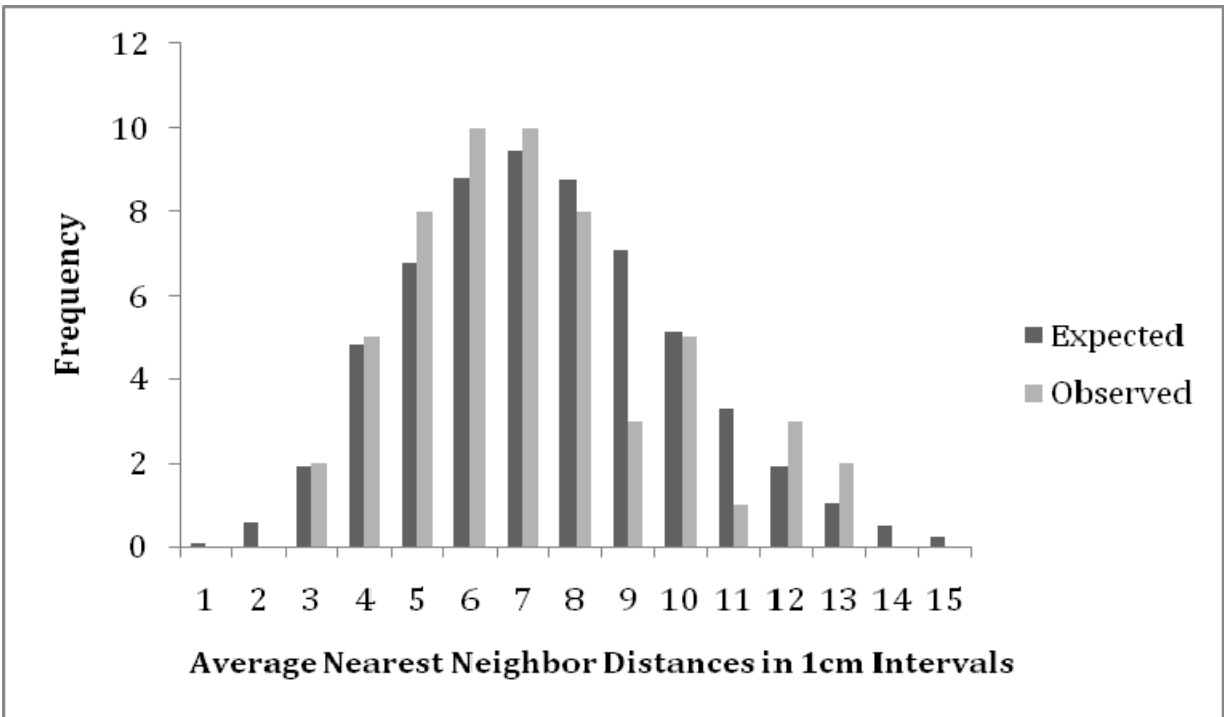
**Figure 14: The effects of density treatment on pit diameter.** Density did significantly affect the average pit diameter ( $F=4.52$ ,  $df=3$ ,  $p=0.009$ ). Treatment four, the high-density situation involving 24 antlions, was significantly different from treatments two and three, the middle-density treatments involving 8 and 16 antlions ( $MS=38.618$ ,  $df=35$ ,  $p=0.0227$ ;  $MS=38.618$ ,  $df=35$ ,  $p=0.0225$  respectively). The high-density treatment did not differ significantly from the low-density treatment, and treatments one (low-density involving 4 antlions), two, and three did not differ from each other.



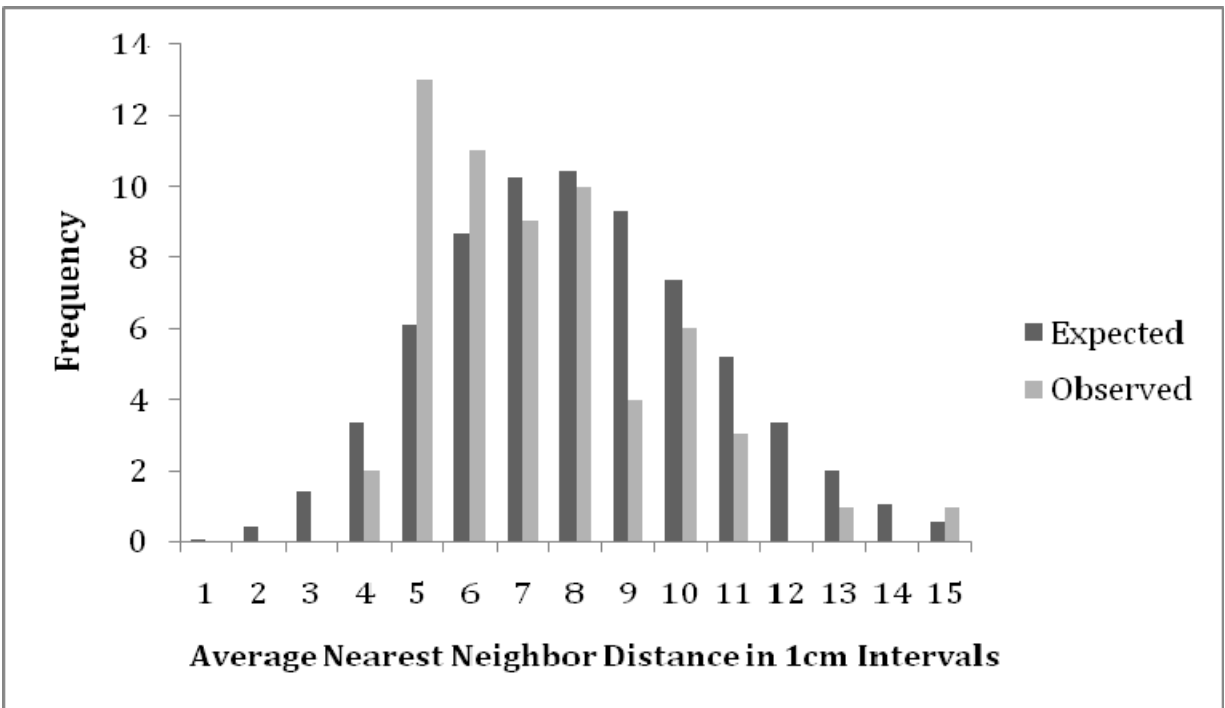
**Figure 15: Treatment one nearest neighbor distance distribution compared to Poisson distribution.** Treatment one did differ significantly from the Poisson Distribution ( $\chi^2=32.463$ ,  $df=17$ ,  $p=0.0132$ ). This suggests that, in low-density situations, antlions survey the situation and choose to maximize the distance between their competition instead of randomly choosing a location.



**Figure 16: Treatment two nearest neighbor distance distribution compared to Poisson distribution.** Treatment two nearest neighbor distances appear more uniform than treatment three, but this may be due to an unrepresentative sample size, with only 24 participants.

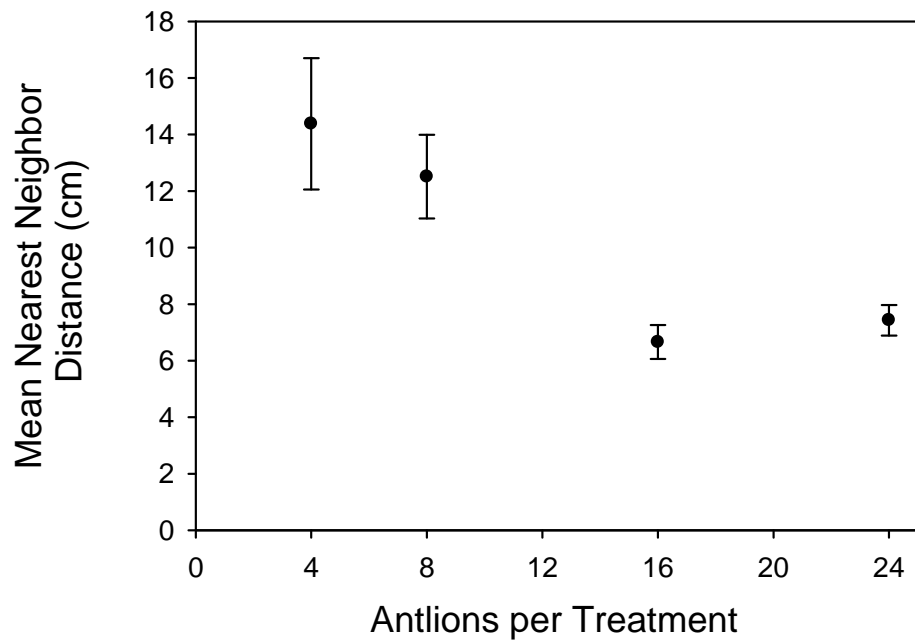


**Figure 17: Treatment three nearest neighbor distance distribution compared to Poisson distribution.** Treatment three distribution of nearest neighbor distances appears to align with the Poisson distribution.



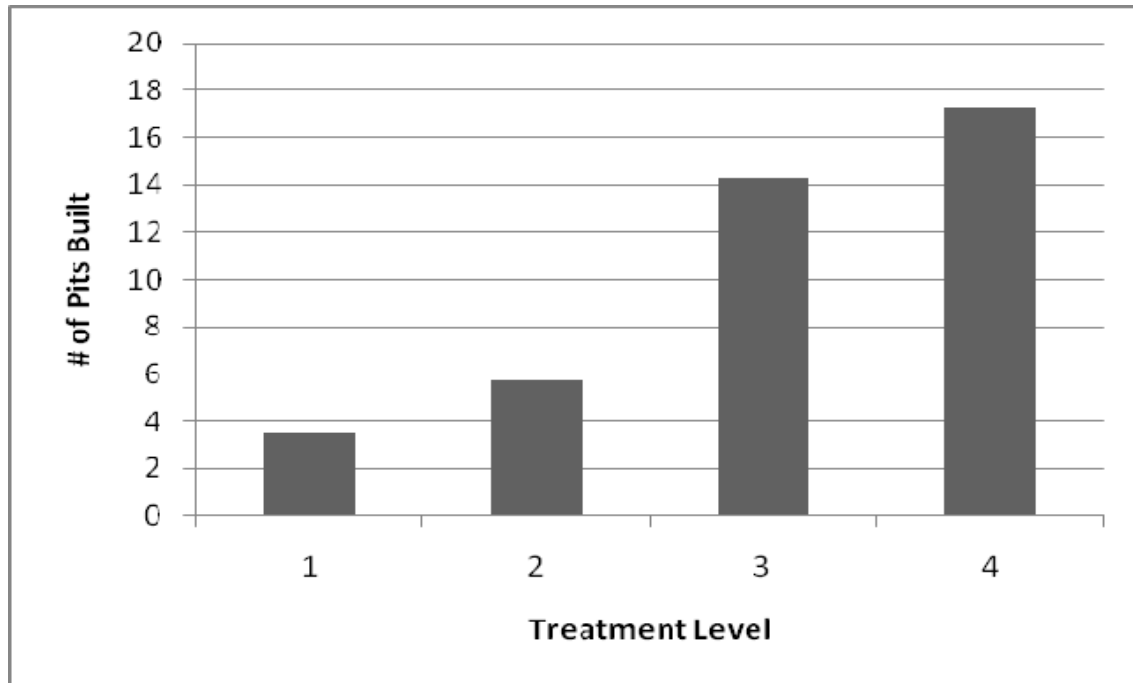
**Figure 18: Treatment four nearest neighbor distance distribution compared to Poisson distribution.** Nearest neighbor distances from treatment four were more uniform than treatment three.





**Figure 19: The effects of density treatment on antlion mean nearest neighbor distance.**

Density treatment level did have a significant effect in decreasing the distance between pits ( $F=14.985$ ,  $df=3$ ,  $p<0.001$ ). Treatments one (4 antlions) and two (8 antlions) did not differ significantly, but that treatment one differed from treatments 3 (16 antlions) and 4 (24 antlions) ( $MS=6.763$ ,  $df=35$ ,  $p<0.001$ ;  $MS=6.763$ ,  $df=35$ ,  $p<0.001$  respectively). Treatment two was significantly different from treatments three and four ( $MS=6.763$ ,  $df=35$ ,  $p<0.001$ ;  $MS=6.763$ ,  $df=35$ ,  $p=0.003$ ). Treatments three and four did not display significantly different nearest-neighbor distances.



**Figure 20: Effects of treatment level on the number of pits built.** The number of pits built did differ significantly between treatment groups ( $G=8.91$ ,  $df=3$ ,  $p<0.05$ ). A trend between low-density and high-density exists, with high-density antlions building more pits.